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RESEARCH ARTICLE

Simultaneous green synthesis of Magnetite-Nanoparticles MNPs using microalgae *Spirulina* sp. for antibacterial activity

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ABSTRACT

Biosynthesize of Magnetic Nanoparticles MNPs is the environmentally friendly synthesis of nanoparticles that can be used as an alternative to commercially available antibiotics. The present study aimed to determine the ability of biosynthesized magnetic nanoparticles of *Spirulina* sp. for antibacterial activity. Microalgae isolated from the Gomaspan river cultured on BG11 medium and, is identified using morphology and molecular method and the optimum growth rate of microalgae studied, the biomass used to synthesize of MNPs then was characterized by a visible color change and Scanning electron microscope SEM, FTIR with XRD. Antimicrobial activity of *Spirulina* sp. and biosynthesize of MNPs. studied using different extracts (ethanol, methanol and Diethyl ether) against growth of *Salmonella Typhi, Streptococcus pyogenes, Escherichia coli* and *pseudomonas aerogenes by* disc diffusion and Minimum inhibitory concentration methods. The antibacterial activity from microalgae *Spirulina* sp. and biosynthesized MNPs from *Spirulina* sp. showed to inhibit growth of bacteria with both methods and the higher inhibition zone showed as (30-37mm). The minimum inhibition concentration showed with ethanol extract (125-500 µg/l). The current study is first report an eco-friendly and convenient method for the synthesis of MNPs using Microalgae *Spirulina* sp. extracts. This biosynthetic process might be useful pharmaceuticals, and medicine treatment of pathogenic bacteria.

Keywords: Nanoparticle, Magnetic, *Spirulina*, Antibacterial, solvent extract

INTRODUCTION

Bacterial infections are still a serious concern in the globe today, because disease-causing bacteria may ultimately evolve methods to resist medications as well. The major public health threat with most of the antibiotics being rendered ineffective in the emergence & spread of multi drug resistant communities as well as the in hospital settings. Clinicians are left helpless with very few alternatives left which are also slipping off their hands (Reygaert 2014). To tackle this menace there is an increasing need to develop newer antimicrobial agents, particularly from medicinal plant extracts that aid in the prevention and treatment of certain diseases (Anwer and Abdulkarim, 2014).

Algae are the most promising resource for new antibacterial. A number of cyanobacteria produce toxins that may have potential pharmaceutical application (Katircioglu et al., 2006). Numerous strains of cyanobacteria have been shown to generate intracellular and extracellular compounds having antibacterial, antifungal, and antiviral properties (Metting and Pyne, 1986; Volk and Furkert, 2006). Organic solvents have been employed to extract the potential lipid soluble active components from microalgae as an effective research technique (Satsry, Rao,1994; Schlege et al., 1999). The antimicrobial substances involved may target various kinds of microorganisms, prokaryotes as well as eukaryotes (Dakshini 1994, Al-Naqshbandy 2000).

The algal metabolism is very versatile, reacting fast to changes in the external environment, and Nekooeietal impart the inhibition of bacterial growth by methanol extracts of benthic Red algae against gram positive and negative bacteria (Nekooei et al., 2021). Malathi et al., (2015) used aqueous, ethanol, methanol. Chloroform, Hexan, crude extract of *Calothrix braunii* against four

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pathogenic fungi and four bacterial species, on the other hand antimicrobial and antifungal activity evaluated by Ali and Doumandji (2017). Brown seaweed *Sargassum swartzii* extracts used by Sujatha and his friends for their antibacterial activity against pathogenic microorganism (Sujatha et al., 2013).

The in vitro investigation of magnetic NPs (MNPs) has introduced modern antibacterial studies into an increasingly attractive research field, in addition to the wide range of exotic nanoparticle (NPs) applications. Since nanosilver particles outperformed nanogold in antimicrobial efficacy, they are advised for use in a variety of antibacterial applications (Aldayel et al.,2022). The size scales from nanometer to micrometer regions contain a large number of microorganisms. Many biocompatible MNPs have been introduced that possess remarkable impacts on various bacterial strains. Conventional synthesis methods such as co-precipitation or hydrothermal techniques have been widely adopted in the production of MNPs (Wei et al., 2015). Depending on the algae species and method of activity, nanoparticles can be synthesized intracellularly or extracellularly (Ponnuchamy et al.,2016). Iron MNPs take advantage over other NPs including viable large scale production, low-cost synthesis and environmental performance security. It may be the bacterial inhibitory action of MNPs (Elwakeel et al., 2018). Shanmuganathan and coauthors (2019) create metallic nanoparticles by using one-step procedure, the metallic salt-containing aqueous solution is sprayed directly over grown microalgal living cells. The objective of current study is to determine ability of *Spirulina* sp. and bio-synthesized of magnetic nanoparticles using *Spirulina* sp. for antibacterial activity.

MATERIALS AND METHODS

Isolation and identification

Microalgal sample was obtained from different sites along the Gomaspan rivers Ebil-Iraq government. Water sample inoculated onto plates of BG11 contain %1.5 agar-agar, incubated at 25°C, pH8 and 2500lux. After two weeks single colony was picked up, and identified morphologically using a light microscope. The species were identified morphologically and by amplification of 16S rDNA.

Determination of dry weight under optimum growth

Algal cultures were cultivated in BG11 broth medium with the optimum growth condition using different pH,Light intensity, and Temperature. The pH was maintained using NaOH and HCl (Adenanetal et al., 2013; Rai *et a.l*, 2015; Zhang et al., 2016). The biomass dried and stored for further use.

Analytical methods

Synthesize of ecofriendly magnetic nanoparticles

Magnetic nanoparticle of microalgae: Iron oxide nanoparticles (MNPs) were synthesized by taking $FeCl_2.4H_2O$ and $FeCl_3.6H_2O$ (2:1 molar ratios) and were dissolved in 50 ml of de-ionized water in a 250ml conical flask heated at 70°C with mild stirring using magnetic stirrer under atmospheric pressure. Then, after 20 minutes, 25 ml of the aqueous solutions of microalgae *Spirulina* sp. was added to the mixture, directly the light yellow color of the prepared microalgae turned to black color. Also, after 20 min, 25 ml aqueous solution of NaOH was added to the mixtures with the rate of 2ml/min for allowing the iron oxide settle-down uniformly. Therefore, the mixture let to cool down at room temperature. Finally, the iron-oxide nano-particles were collected by decantation to form magnetite nano-particles. Moreover, the magnetite formed were washed using deionized water and kept in dissector for later use the synthesized MNPs characterized by Fourier transform infrared (FT-IR) analysis, the morphology observed by Visual and Scanning electron microscope (SEM) the purity and crystalline of metallic nanoparticles identified by X-ray diffraction spectra(XRD)-spectra (Ponnuswamy et al., 2013; Hawezy et al., 2020, Hamadamin et al. 2022).

Preparation of micro algal extracts

20grm of dry biomass successively extracted with 200ml of 96% ethanol, diethyl ether and methanol using soxhlete extractor for 4hrs. Benedethi et al. 2018. After that, the solvent was removed by incubation at 60 °C. (50-1000 µg/ml) and kept at 4 °C for future use.

Antibacterial activity

Diffusion method

Bacterial cultures *Salmonella Typhi*, *Streptococcus pyogenes*, used in the study purchased from Media Diagnostic Center – Erbil/Iraq, *Escherichia coli* and *Pseudomonas aerogenes* obtained from microbiology lab College of Health Sciences. The bacterial culture incubated on Muller Hinton agar with 500µl from each extract by using well diffusion methods then incubated at 37 °C for 24hrs. The inhibitory zones were measured and the results of an antibacterial activity were compared to the control according to National committee for Clinical Laboratory Standards (NCCLS) medium without MNPs used as negative control Reflacin and Penicillin G used as positive control(Abdo et al., 2013).

Minimum inhibitory concentration (MIC)

Different volume 50-1000 µg/ml of each algal extracts without nanoparticles and with algal extracts with MNPs were added to the tubes and incubated at 37°C for 24 hrs. The tubes that showed no growth with lowest concentration selected as MIC (Salvador et al., 2007).

Statistical analysis

The experiment's results were statistically evaluated with SPSS version (28) and Microsoft Excel Office 2010. A probability value of P = 0.05 was declared significant by the significant correlation difference test, and one way ANOVA was used.

RESULTS AND DISCUSSIONS

Isolation and identification

In the present study antibacterial activity of microalgae *Spirulina* sp. was examined using different solvent extracts and biosynthesized nanoparticles. After the purification of microalgae, growing filaments were observed under light microscope and photographed. The filaments were spiral, length (typically 100–150 microns) and with a diameter close to7-9 microns was identified as *Spirulina* sp.as showed in Fig. 1

The sequence from 16S rDNA of algae specimen Fig. 2 was made of 1200-1400.and was identified as *Spirulina* sp.as described in previous report (Abdulkareem and Anwer 2021).

Effect of pH, temperature and light intensity on growth of microalgae

In current study the optimum temperature for the growth of microalgae *Spirulina* sp obtained at 28 °C and the lowest growth found at 20 °C which shows denaturized of pigment after 10 days of cultivation. This result shows that increasing of temperature from 20 to 30 °C caused of increasing of growth rates (cell fresh & dry weight) but below this value caused decreasing of (cell fresh with cell dry weight). This result is agreeing with finding of Dhargalkar 2004, the microalgae showed growth at all pH values, the best growth determined at pH 8. with light intensity 2800 lux. Fig. 3 this pH was related with the pH of isolated place, Moreno and his friends illustrate that positively affected by light intensity (Moreno etal 2019).

Characterization of nanoparticles

The green colour of MNPs showed as a black colored within a temperature range of 80°C Fig. 4. Photosynthesis and respiration are both important for decreasing metallic ions in algae, resulting in the formation of metallic nanoparticles within the cells. Gahlawat, Choudhury, 2019 and Mukherjee and colleagues (2021) observed that studying the manufacture of metallic nanoparticles by algae can lead to phyco-nanotechnology, a new branch of nanotechnology. The scanning electron microscopy (SEM) is an appropriate tool for resolving individual MNPs and the structure of their linked Nano trusses. The SEM image of the synthesized magnetite nano-particles is shown in Fig. 5 it is investigated that The magnetite nanoparticles are agglomerated with a spherical shape and narrow size distributions and generated in vast quantities, with average-particle sizes of about 52.05-55.98. The images of the prepared nanoparticles have been taken in different modulation, the presence of agglomeration is clarified in terms of magnetic dipole-interactions between the nano-particles (Huang et al, 2010; Salih et al, 2017; Ahmed et al, 2018; Nsar et al 2019).

The FTIR spectrum of metallic nanoparticles is shown in Fig. 6.Where the vibrations at 3500-3560cm-1 were attributed to the OH stretching of amino acids and carbohydrates additionally of the presence of alcohols and phenols. The results of the FT-IR examination revealed that the presentation of the FT-IR characteristic spectrum was excellent. The iron oxide nanoparticles had been successfully biosynthesized, as seen by the several well-explained peaks at 577, 631, 991, 1631, and 3431 cm1. Due to the presence of iron–oxygen FeO, two peaks at 577 and 631 cm1 were observed, indicating that the produced nanoparticles are iron–oxide. Furthermore, the arrival of the NO3 group results in the appearance of a little peak at 991. The peaks at 1631 cm1 and 3431 cm1 are caused by the absorbed vibration of H_2O, as well as the surface-hydroxyl and Hydroxide stretching modes. In bacteria connected to seaweed, several novel antibiotic-active compounds have already been found (Martin et al., 2014). Dell Anno and his friends (2000) demonstrated that the extracted algae can be used as feed additive to improve the gut health.

The phase purity and crystalline of metallic nanoparticles (biosyn-$Fe_3O_{4)}$ identified by XRD-spectra (Fig. 7). The

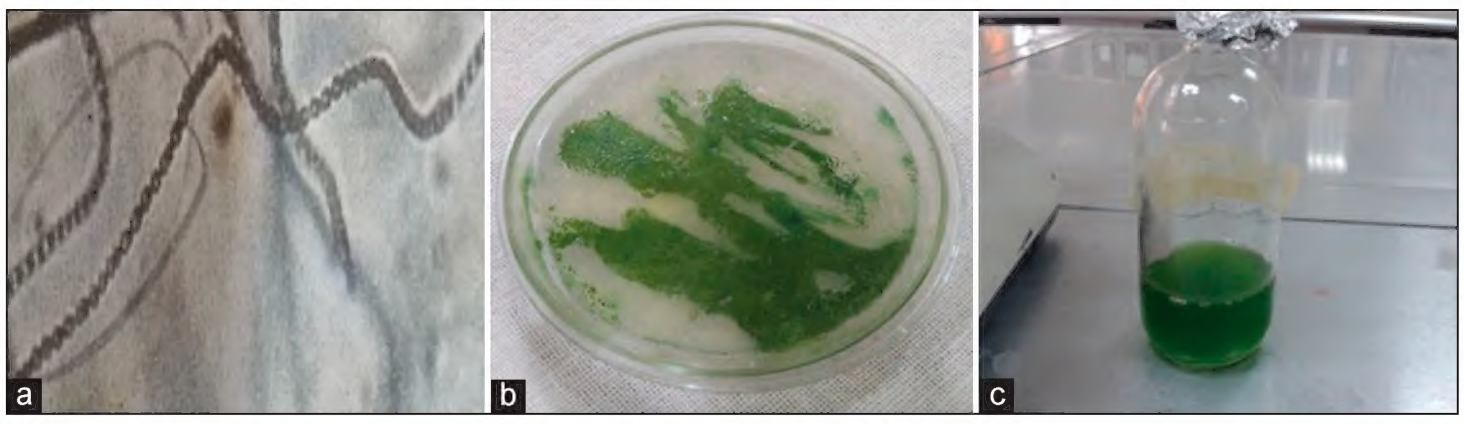

Fig 1. (a) *Spirulina* sp. under microscope (b) S*pirulina* sp. in BG-11 agar (c) in BG11 broth.

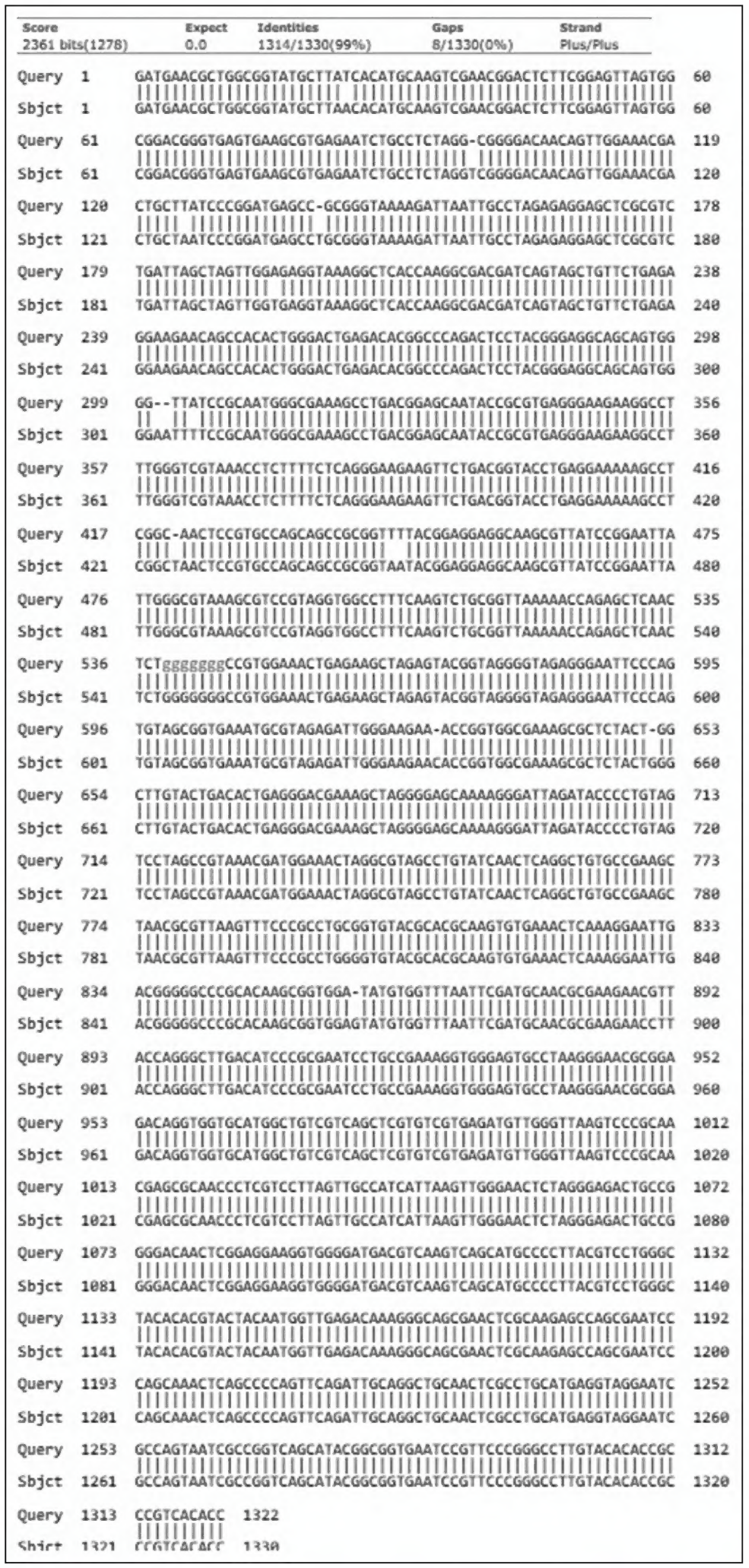

Fig 2. Pair wise alignment of 16S rDNA sequence of Spirulina sp. Query is the study or sample sequence and Sbjct is the GenBank sequence.

crystallite mean-diameter determined by the diffractogram using the formula above is 45 nm, which is consistent with the size seen in the above electron micrographs. The high intensity of these peaks also supported substantial X-ray scattering in the crystalline phase.

Antimicrobial activity

Solvent extracts of Spirulina sp. were tested against bacteria *Salmonella Typhi, Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aerogenes*. Antibacterial activities were determined by using well diffusion method as shown in Table 1. The extracts (ethanol, methanol, and diethyl ether) had various degrees of antibacterial activity against the pathogenic microorganisms that were tested, the diameter of inhibition zone against *Salmonella Typhi* by using algal extracts were 27,26,24 mm for *Streptococcus pyogenes* 28,14,19 mm for *Escherichia coli* 31,31,30 mm and for *Pseudomonas aerogenes* inhibition zone was 21,17,18 mm respectively and the strongest activity showed against *E.coli* during using all solvent extracts the maximum inhibition zone showed with ethanol extract (21-31 mm). In order to examine the antibacterial activity of metallic nanoparticle of *Spirulina* sp. disc diffusion and minimum inhibitor concentration; inhibition zone observed around the disc, the biosynthetic *Spirulina* sp. showed ability to inhibit growth all type of bacteria the most effective bacteria were *Escherichia coli* and *Streptococcus pyogenes* with ethanol extract (32-37 mm. The inhibition zone of MNPs from *Spirulina* sp. was *Salmonella Typhi* 32 mm, *Streptococcus pyogenes* 30 mm, for *Escherichia coli* 37 mm and for *Pseudomonas aerogenes* inhibition zone was 33mm and the controls showed low antibacterial activity. In study done by Ibrahim et al 2016 showed that the anti-microbial activity of AgNPs inhibit growth of both gram positive *Staphylococcus aureus, Bacillus subtillis,* and *gram negative Salmonella spp., Escherichia Aboud bacteria* significantly. Abboud and colleagues (2014) found that copper oxide nanoparticles (CONPs) produced using brown alga extract exhibited maximum antibacterial activity against two different strains of bacteria *Enterobacter aerogenes and Staphylococcus aureus.* Karakurt and co-authors (2014) studied the antibacterial activity of immobilized samples against *Escherichia coli and Staphylococcus aureus* bacteria strains and they found that the combination shows no synergistic impact on antibacterial activity.

During the studding of the minimum inhibitory concentration (MIC) Table 2, different dilutions were used to evaluate the inhibitory effect of solvent extract and MNPs on the growth of four pathogenic bacteria *Salmonella Typhi, Streptococcus pyogenes, Escherichia coli, Pseudomonas aerogenes.* The results showed that *Spirulina* sp. inhibited bacterial growth with all solvent extracts (Ethanol, Methanol and Diethyl ether) with the minimum(MIC) inhibition concentration showed with ethanol extraction 125-500 µg/ml when MNPs using *Spirulina* sp. used for MIC obtained at the concentration of 50-125 µg/ml, the solvents without *Spirulina* used as control and was ranged between 500-1000 µg/ml. Biosynthesis of metallic nanoparticles employing a variety of biological agents has a number of benefits over chemical synthesis. as well as mechanical synthesis

Table 1: Antibacterial activities of different extracts of *Spirulina* sp. zone of inhibition in mm

Antibacterial extracts and control	*Salmonella Typhi*	*Streptococcus pyogenes*	*Escherichia coli*	*Pseudomonas aerogenes*
Ethanol extract	27	28	31	21
Methanol Extract	26	14	31	17
Diethyl Ether extracts	24	19	30	18
Spirulina -Fe_3O_4-NPs	32	30	37	33
Reflacin	23	20	21	13
Penicillin G	16	30	16	25

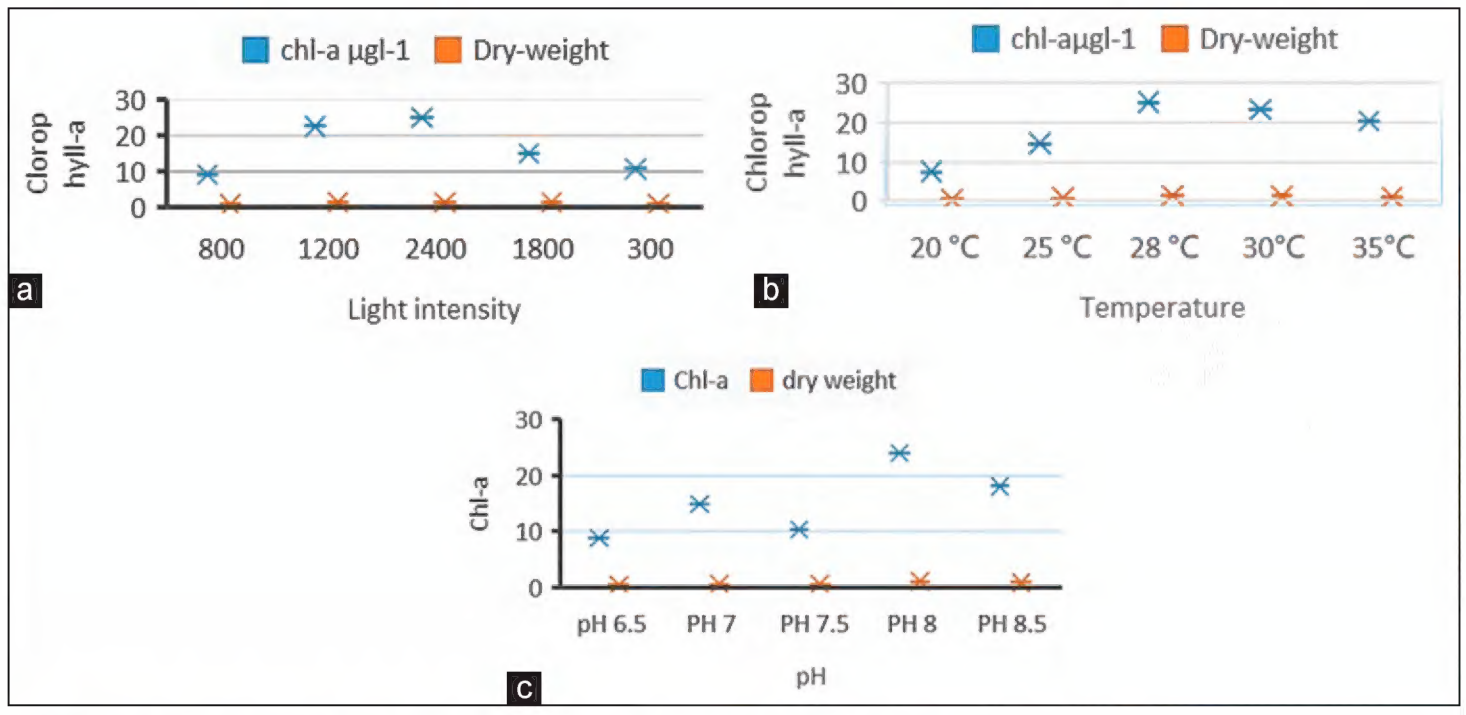

Fig 3. Optimum growth of microalgae obtained at (a) pH 8-24, (b) 28 ℃, (c) 1800 lux).

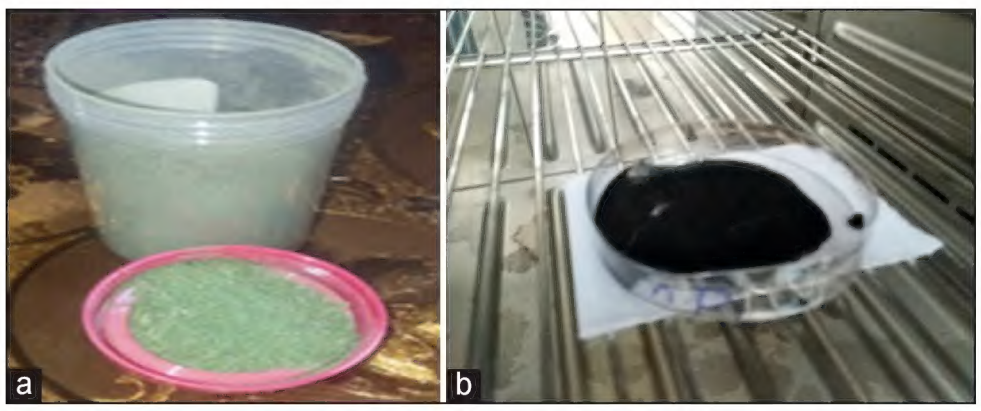

Fig 4. *Spirulina* biomass (a) and MNPs from *Spirulina* sp (b).

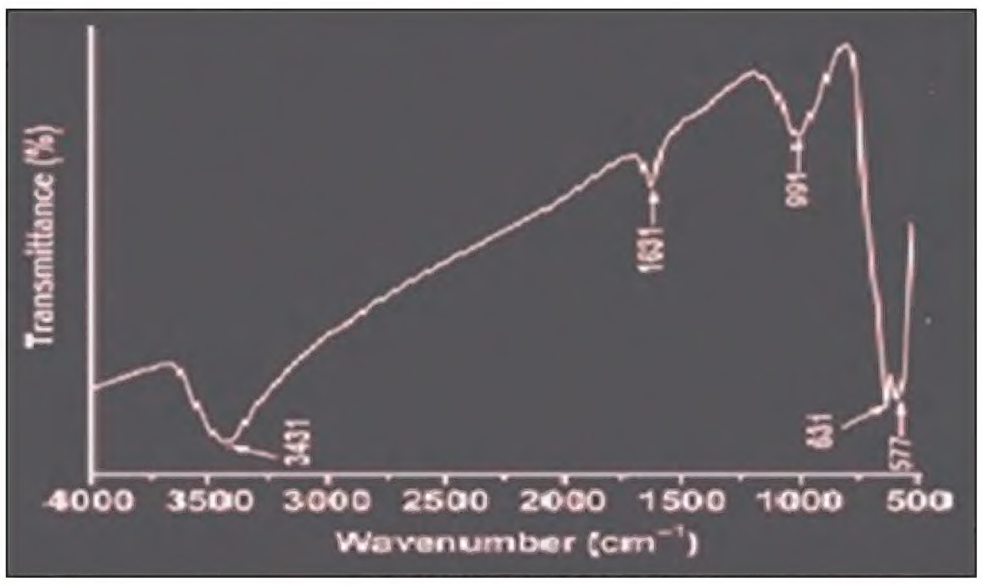

Fig 6. FTIR spectra of biosynthesized MNPs from *Spirulina* sp.

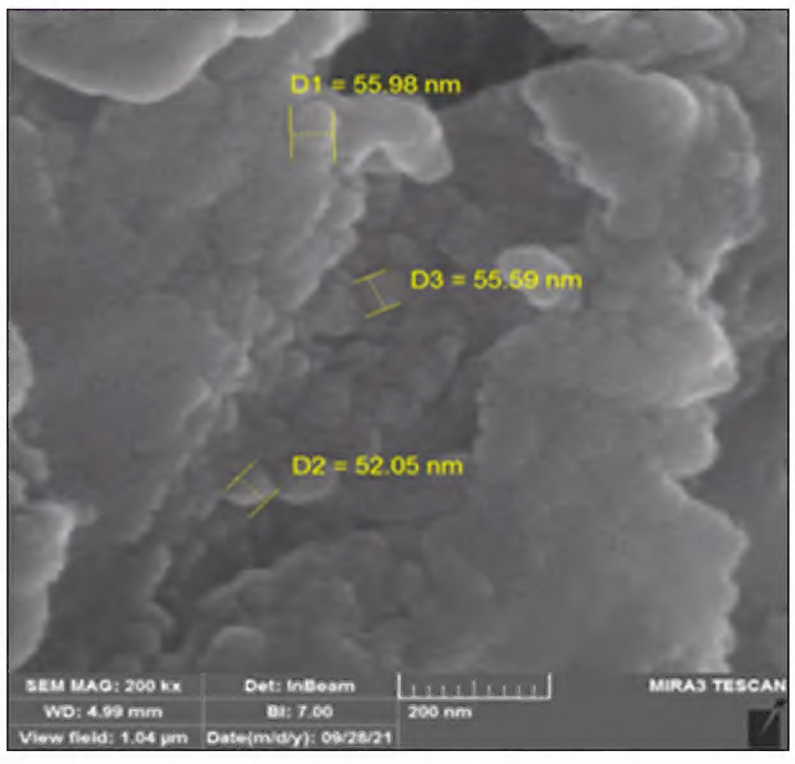

Fig 5. SEM monograph of magnetic nanoparticles from *Spirulina* sp.

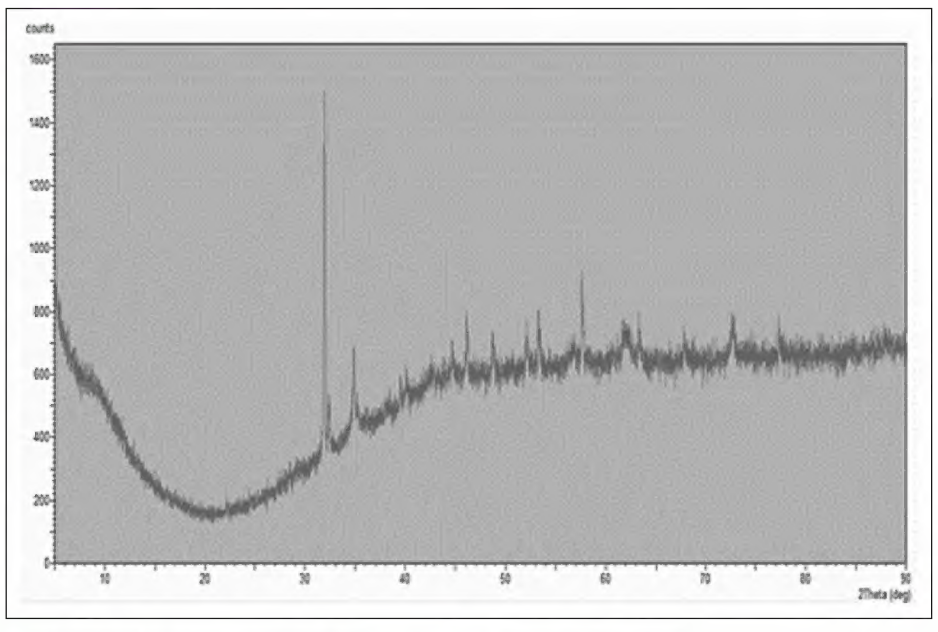

Fig 7. XRD-patterns of the magnetite nanoparticle.

Table 2: Antimicrobial screening of *Spirulina* sp. extracts determined by MIC in μg/ml)

Bacterial strains	Minimum inhibitor concentration (MIC) μg/ml			
	Spirulina sp. Ethanol extract	Spirulina sp. Methanol extract	Spirulina sp. Diethyl ether	Spirulina sp. Fe_3O_4-NPs
Salmonella Typhi	500	1000	750	100
Streptococcus pyogenes	125	500	250	50
Escherichia coli	125	250	250	50
Pseudomonas aerogenes	250	500	500	125

Table 3: Antimicrobial activity between different extractions by one-way ANOVA

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Salmonella					
Between Groups	1767500	3	589166.667		-
Within Groups	0	12	0	-	
Total	1767500	15			
Streptococcus					
Between Groups	466875	3	155625	-	-
Within Groups	0	12	0		
Total	466875	15			
Escherisia					
Between Groups	116875	3	38958.333	-	-
Within Groups	0	12	0		
Total	116875	15			
Pseudomonas					
Between Groups	421875	3	140625	-	-
Within Groups	0	12	0		
Total	421875	15			

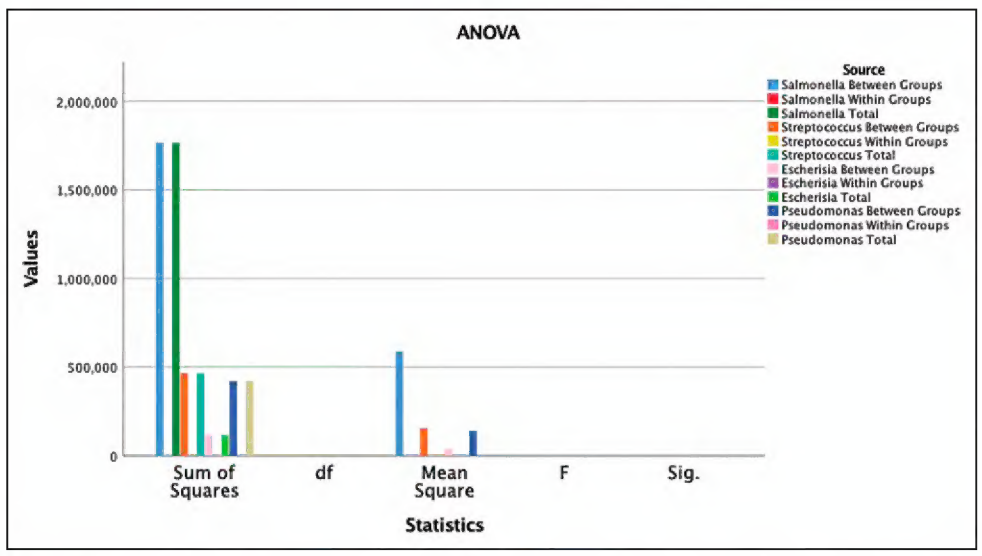

Fig 8. Antimicrobial activity between different extractions by one-way ANOVA.

techniques. Furthermore Yoshimura et al., 2019 illustrated in their study that the effect of SNPs to growth of bacteria is related to breaking down of bacterial DNA molecules Negi et al (2013) and Ibraheem et al. (2016) showed that SNPs, even at concentrations below MIC value, can reduce expression level of alpha hemolysin and inhibit growth of *Staphylococcus aureus*. Mohammed et al (2021) conducted that Ag_2O nanoparticles have the way for a new generation of antibacterial agents against the emerging multidrug resistant pathogens.

The statistical analysis showed that there was statistically significant difference between different extraction $p > 0.05$ Fig. 8 and Table 3. The developed nanoparticles were characterized by SEM, and FTIR measurements and showed antibacterial activity, showed significant antimicrobial activity against *Salmonella Typhi Streptococcus pyogenes, Escherichia coli* and *pseudomonas aerogenes*.

CONCLUSION

Biosynthesize of MNPs is the environmental friendly synthesis of nanoparticles which can be used as an alternative to commercially available antibiotics the current study is the first to describe an environmentally friendly and practical approach for synthesizing MNPs from Microalgae *Spirulina* sp. extract. This biosynthetic process could be useful in a variety of disciplines, including biotechnology, medicines, and the treatment of pathogenic microorganisms in medicine.

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Author contribution

All Analysis, manuscript design and the computational framework with data analyze were provide by the author.

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